REMARKS

Pursuant to 37 CFR 1.111 reconsideration of the Office Action dated February 29th, 1990 is hereby respectfully requested.

Relying on 35 U.S.C. 112, first paragraph, the Examiner has rejected claims 75 to 90 and 93 to 94 as not based on an enabling disclosure. Applicants respectfully traverse the rejection reconsideration.

The Examiner contends that the disclosure is non-enabling in respect of non-mammalian eukaryotic cells. The Examiner expects the machinery of gene amplification to be cell-specific.

It is not entirely clear whether the Examiner considers Applicants' arguments, as set out in the Amendment filed on November 24th, 1989 to the Office Action dated May 24th, 1989, not persuasive because she considers that GS DNA amplification per se has not been shown to occur in non-mammalian cells or because Applicants have not shown that GS can function as a selectable marker in non-mammalian cell types. Clarification is respectfully requested. In either case, however, Applicants respectfully submit the Examiner's objections to be ill-founded.

In the first case, Applicants respectfully direct the Examiner's attention to the Donn et al. document cited in the

International Search Report issued in respect of the instant application. Donn et al. disclose the selective amplification of the endogenous GS gene in plant (alfalfa) cells. Administration of L-phosphinothricin (L-PPT), a mixed competitive inhibitor of GS, to a suspension of alfalfa cells leads to a three to seven-fold elevation in GS enzyme levels. This elevation is found to be due to a four to eleven-fold amplification of the genomic GS gene and a concomitant eight fold increase in steady-state GS mRNA levels. The toxic effects of L-PPT are thereby overcome. This experimental system closely mirrors the MTX - induced amplification of genetic DHFR genes in mammalian cells, and the MSX-induced amplification of the GS gene in CHO cells as described in the Sanders et al. documents cited by the Examiner. It is therefore known that non-mammalian cells possess the machinery required to amplify the GS gene.

Applicants cannot point to a reference showing GS amplification in yeast. However, given that amplification of other types of DNA is well known in yeast, Applicants see no reason to suppose that the GS gene could not be amplified. The Examiner is respectfully referred to Kabak, D. et al, Bacteriol (1978) 134, 237-245, a copy of which is enclosed and listed on Form PTO-1449 for the Examiner's convenience. This article clearly demonstrates gene amplification in

yeast, in this case genes coding for ribosomal RNA. Certain strains of Saccharomyces cerevisiae are monosomic for chromosome 1, which encodes 50 to 70% of the ribosomal RNA genes. These strains contain about 25% fewer rRNA genes than diploid strains. When these strains are repeatedly subcultured, colonies are isolated that have magnified their number of rRNA genes to the diploid amount while remaining monosomic for chromosome I. Kabak, D. et al demonstrate that the generation of these colonies having increased rRNA gene levels is due to the creation of heterozygous gene clusters localized to a few chromosomes, in other words to DNA amplification rather than chromosome duplication or the like.

Applicants therefore respectfully submit that DNA amplification is well known in yeast, and see no reason to doubt that the instant invention could be worked both in yeast cells, and, as evidenced by the Donn et al. article, in plant cells.

From the Examiner's Actions, Applicants note a suggestion that the Examiner considers DNA amplification to be a highly specific event which is the property of certain cell types. This is not the case. DNA amplification is an intrinsic function of all living cells. In this instance, the Examiner is respectfully referred to Vel'Kov, Soviet Genetics (1982) 18, 348-396, a copy of which is enclosed and

listed on Form PTO-1449 for the Examiner's convenience. In the abstract of Vel'Kor, it is clearly stated that gene amplification is a universal mechanism for all classes of organism. The Vel'Kov article reviews DNA amplification in bacteriophages, bacteria, yeasts, Drosophila, animal viruses and animal cells. The conclusion (page 394, lines 13-16) is that DNA amplification is a widespread phenomenon, occurring when gene products are the limiting factor in the growth of the organism, and is common to all cell types.

Although it is not at present clear how, in detail, a particular gene is amplified, Applicants respectfully submit that, based on the evidence of the documents cited above, it is evident that a wide variety of cell types possess the machinery to amplify genes.

Thus gene amplification is an intrinsic function of living cells. Genes are spontaneously amplified and cells containing amplified gene copies have either improved or diminished prospects of survival dependant upon the prevailing growth conditions, environment etc., i.e. if the gene product is the limiting factor in the growth of the organism, cells containing amplified gene copies will have a survival advantage over unamplified cells and culturing will lead to selection of amplified cells over unamplified cells. For example, in the case of a gene coding for antibiotic

resistance, cells having amplified copies of the gene will have a survival advantage and be selected over cells only possessing a single copy of the gene when cells are cultured in progressively elevated levels of antibiotic. The principle is exactly the same in the case of GS where elevated levels of an inhibitor, e.g. MSX, are used to select for cells in which the GS gene has been amplified. Given that it is established that gene amplification is a universal mechanism for all cell types, including eukaryotic cell types, it is reasonable to predict that the GS selection/amplification system of the present invention will be applicable to eukaryotic host cells in general.

If the Examiner's point is the second one identified above, Applicants likewise submit, given that GS functions as a selectable amplifiable marker even in GS+ mammalian cells, that it is reasonable to predict that GS should also do so in other eukaryotic cells.

Gene amplification is a universal mechanism in eukaryotic and other cells. It is known that endogenous GS genes amplify in different eukaryotic cell types, and likewise that an exogenous GS gene may be amplified in preference to the endogenous gene in typical mammalian cells, i.e. CHO cells. Thus it is reasonable to predict that GS could be used as a dominant selectable/amplifiable marker in

any eukaryotic cell type. Modifications and minor adjustments might be required in specific cases. However, Applicants submit that any such modifications or adjustments are well within the compass of, and would be merely routine for, workers skilled in the art.

In view of the reasons discussed above, applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 75 to 90, and 93 to 94 under Section 112, first paragraph.

The Examiner has rejected claims 75 to 95 under 35 U.S.C. 112, second paragraph, as being indefinite. In response to the Examiner's helpful suggestions, claims 75, 83, 85, 91, 93, 94 and 95 have been amended. Applicants respectfully request the Examiner to reconsider and withdraw the rejection of the claims under Section 112, second paragraph.

Relying on 35 U.S.C. 103 the Examiner has rejected claims 95 to 97 as obvious under Sanders et al. in view of Pennica et al. The Examiner also points out that it was known for two genes to be incorporated into one plasmid. Applicants respectfully traverse this rejection and request reconsideration for the following reasons.

Sanders et al. provide evidence to show that the endogenous GS gene has been amplified in a MSX-resistant CHO

cell line. In the course of accumulating this evidence, Sanders et al. cloned what would appear to be only a partial cDNA copy of the CHO GS gene. Sanders does not teach further in respect of GS or the GS gene.

Pennica et al. isolated a human tPA cDNA from a cDNA library. In order to provide a full length clone, they isolated a genomic tPA clone and used this to probe for amino terminal-specific tPA cDNA clones. They therefore suggest a method for obtaining full-length cDNA clones. Subsequently, Pennica et al. express the full-length tPA cDNA in E.coli under control of the bacterial trp operon.

The Examiner contends that it would be obvious to use the teachings of Pennica et al., who disclose a method for the recovery of a full-length cDNA, in order to obtain a full-length GS cDNA from the partial clone of Sanders. Applicants respectfully submit that in raising this rejection, the Examiner appears to have inadvertently overlooked the fact that the plasmids claimed in claim 95 of the present application comprise both a GS gene and a gene encoding a desired heterologous peptide.

It is respectfully pointed out that neither Sanders et al. nor Pennica et al. is in any way concerned with amplification of an exogenous gene coding for a desired protein. It is therefore clear that neither reference is

relevant to the present invention. The plasmid as claimed in claim 95 is designed to enable amplification of the gene for the desired protein. In the absence of any teaching regarding such amplification, it cannot be seen how any combination of the two citations can render the present claims obvious.

It is submitted that even if Pennica et al. and Sanders et al. were combined then the logical combination would be to use the plasmid designed by Pennica et al. to isolate a full length copy of the GS gene being investigated by Sanders et al. The logical combination of two references would not have lead to the present plasmid, and would not have rendered the features of the present invention obvious to a person of ordinary skill in the art at the time applicants made their invention.

It is respectfully submitted that there is no incentive in either Pennica et al. or Sanders et al. for a skilled person to place on the same plasmid a GS gene and a tPA gene. Neither of the two references even remotely suggest that this would be worth doing. Thus, it is respectfully submitted that the subject matter of the claims would not have been obvious at the time the invention was made to one of ordinary skill in the art aware of the disclosures of Pennica et al. and Sanders et al.

Applicants respectfully point out that the issue is not whether or not it would have been possible, at the time, to carry out the instant invention. The issue is whether the invention was, at the time, obvious. Applicants acknowledge that the incorporation of two genes into one plasmid was known. Thus, the skilled person could have produced a plasmid according to claim 95. It is respectfully submitted, however, that no combination of Pennica et al. and Sanders et al. provides the incentive which makes it likely, much less inevitable, that a skilled person would have produced such a plasmid. There is nothing which could possibly lead a skilled artisan to suppose that the combination of the GS gene and a gene encoding a desired protein would lead to improved expression of the latter gene under appropriate conditions. No skilled worker would be made aware by these two references that the GS gene could be employed as a selectable and amplifiable marker gene, and would therefore not be led to the construction of a plasmid such as that claimed in claim 95. Applicants therefore submit that the present invention is clearly and substantially unobvious under Pennica et al. and Sanders et al.

Furthermore, claims 96 and 97 concern vectors containing either a specific GS minigene, or an SV40-GS transcription unit. Applicants have found that both the minigene and the

transcription unit have desirable properties, as is taught in the specification of the present application. The Examiner's attention is respectfully directed to pages 28 and 29 of the specification. Neither Pennica et al. nor Sanders et al. make any reference whatsoever either to such minigenes or to SV40-GS transcription units. Therefore, Applicants respectfully submit that claims 96-97 are not obvious under Pennica et al. and Sanders et al.

For the reasons set out above, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 95 to 97 under Section 103.

In view of the foregoing, it is respectfully submitted that all of the Applicants' claims are in condition for allowance and such notice is requested. The Examiner is respectfully requested to call the undersigned attorney if any minor matter remains.

Respectfully submitted,

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